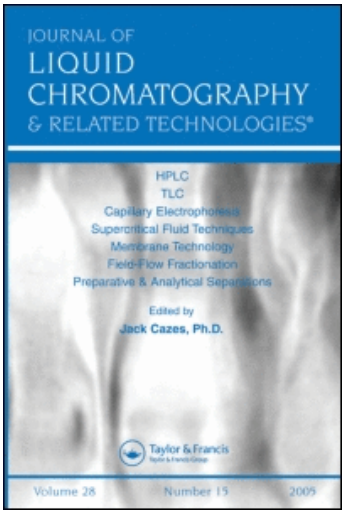

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DETERMINATION OF BLOOD GLUCOSE BY QUANTITATIVE TLC ON PREADSORBENT SILICA GEL PLATES

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ABSTRACT

A thin layer chromatography method was developed for quantification of glucose in human blood serum using preadsorbent silica gel plates, acetonitrile-water (85:15) mobile phase, methanolic sulfuric acid detection reagent, and scanning densitometry. Results are reported for the measurement of glucose by the TLC method before and after consumption of a sucrose solution. The method was validated by determining the precision for replicate analyses and accuracy by analyzing a spiked sample and also comparing results with those given by a commercial kit intended for use by diabetics for home monitoring of glucose levels. The TLC method is simple to perform and can serve for the analysis of other sample types and as an interesting experiment illustrating quantitative TLC in college analytical chemistry or biochemistry laboratory courses.

INTRODUCTION

Diabetes results from a deficiency in the secretion or action of insulin, a hormone that regulates the metabolism of sugars. The analysis of blood glucose level is essential in the diagnosis and treatment of diabetes.

Upon ingestion of sugar, blood glucose rises only slightly in normal individuals, whereas diabetics experience increases to dangerous levels. Hyperglycemia is characterized by a blood glucose level greater than 140 mg/dL.

A method described[1] for the thin layer chromatography (TLC) separation and detection of sugar standards was modified to allow quantification of glucose in blood by scanning densitometry. The proposed method was used to measure blood glucose levels from a non-diabetic individual before consumption of a sucrose solution and at 30 min intervals for 1.5 hr after consumption. The precision of the method was evaluated by analyzing replicate samples, and accuracy was determined by analysis of a sample before and after spiking with a known concentration of glucose and by comparing results with those from a home glucose monitoring kit purchased in a pharmacy.

EXPERIMENTAL

Preparation of Standards and Samples

Blood was taken from a healthy female college student by using a lancet to prick a finger. The first drop was discarded and ca. 80 μL was collected in an eppendorf tube. The blood was centrifuged and the serum or liquid portion was analyzed by TLC.

Anhydrous ACS reagent grade glucose was purchased from Sigma (St. Louis, MO) and the following four TLC standard solutions were prepared in HPLC grade water: 0.220, 0.440, 1.10, 2.19 μg/μL.

Thin Layer Chromatography

TLC was carried out using the mobile phase specified in the earlier paper,[1] acetonitrile-water (85:15), with an ascending development distance of 16 cm beyond the preadsorbent-silica gel interface in a paper-lined Camag (Wilmington, NC) twin-trough tank. Development required ca. 90 min. Instead of the recommended[1] 20 X 20 cm Whatman (Clifton, NJ) K5 silica gel plates, Whatman LK5DF silica gel plates, containing a preadsorbent zone, were substituted to simplify application of sample and standard solutions and with 19 lanes to facilitate densitometric scanning of the glucose zones. Another change from the earlier procedure[1] was that layers were developed once rather than using four ascents with drying in between. The layers were pre-cleaned before initial zone application by development to the top with dichloromethane-methanol (1:1), followed by drying in a fumehood.

Twelve μL of each standard solution (2.64-26.3 ug) and duplicate 10.0 μL aliquots of blood samples were spotted on the preadsorbent areas of adjacent lanes using a Drummond (Broomall, PA) 25 μL digital microdispenser. The initial zones were dried with warm air from a hairdryer prior to insertion into the tank. After development, the plate was dried with a hairdryer and zones were detected by spraying with sulfuric acid-methanol (1:3) and heating at 110°C for 15 min in an oven.

The glucose standard and sample zones were scanned at 420 nm with a Shimadzu (Columbia, MD) Model 930 densitometer in the single beam-single wavelength visible reflectance mode using a slit size of 6 mm (height) x 0.4 mm (width). This wavelength of maximum absorption was identified by measuring the in situ spectrum of a glucose standard zone using the spectral mode of the densitometer. For each plate, a calibration curve was calculated from the standard zone areas and weights (μg) using a PC linear regression program, and the weights (μg) of glucose in the duplicate 10 μL sample zones were interpolated from the curve. The concentration of glucose in blood was converted by calculation to mg/dL.

Analysis with the Blood Glucose Monitoring Kit

To use the monitoring kit to determine blood glucose, the finger tip was lanced and the first drop discarded. Blood was then applied to a pink square on a test strip, and the test strip was inserted into the glucose meter, which gave the glucose concentration. Application of blood to the test strip resulted in a chemical reaction that yielded a blue color; higher levels of glucose produced a darker blue color. To ensure correct results from the blood glucose meter, a control solution containing a known amount of glucose was applied to a test strip and read prior to measurement of unknowns.

RESULTS AND DISCUSSION

Glucose formed a brown-black circular zone with an R_f value of 0.31 when developed on the K5 preadsorbent silica gel plate with acetonitrile-water (85:15). The calibration curve had a linearity correlation coefficient (r) value of ca. 0.997 and consistent slope and intercept values from plate to plate.

Blood glucose levels were analyzed in duplicate prior to consumption (0 min) of 41 mL of water containing 14.25 g of sucrose and again at 30, 60, and 90 min after consumption using the TLC method and the monitoring kit. The following average concentrations (mg/dL) were found: 0 min-124 (TLC), 122 (kit); 30 min-115, 121; 60 min-140, 141; 90 min-125, 133. These data show that after 60 min

Table 1

R_f Values of Sugars on Preadsorbent Silica Gel Plates Developed with Acetonitrile-Water (85:15)

Sugar	R_f
Sucrose	0.25
Glucose	0.31
Trehalose	0.18
Mannose	0.34
Galactose	0.27
Xylose	0.47
Ribose	0.48
Lactose	0.17
Raffinose	0.090
Fructose	0.33
Melezitose	0.15
Maltose	0.19

there was an increase of about 20 mg/dL followed by a decrease of about 8 mg/dL after 90 min. The percent difference between the TLC and kit results at each time period ranged from 0.7 to 6.2% with a mean of 3.4%. The percent difference between the duplicate TLC results ranged from 1.6 to 14%, with a mean of 7.9%.

To further validate the precision of the TLC method, a 200 μL blood sample was collected from another subject and analyzed four times. An average glucose value of 137 mg/dL was obtained with a relative standard deviation of 3.3%. The concentration of glucose was increased by 50% by adding 6.85 μL of a 10.0 μg/μL standard solution to 100 μL of the analyzed serum, and the spiked sample was reanalyzed in duplicate to further test accuracy. After correcting for dilution of the sample by the spike solution, the average concentration was found to be 201 μg/μL, representing 97.8% recovery of the spike.

Although sucrose is broken down in the body to produce glucose and fructose in a 1:1 ratio, only a single zone of glucose was detected in sample chromatograms. This is most likely due to slow absorption of fructose in the intestine, which did not allow fructose to enter the blood stream in the time periods over which the samples were analyzed. Zones of fructose and glucose are readily separated in the TLC system (Table 1), and an advantage of the TLC method is that both of the sugars could have been quantified if they appeared in the blood samples. The method would also be applicable to the simultaneous determination of other sugars (Table 1)

in a variety of samples such as foods or biological samples, from which sugars can be extracted with 70% ethanol,[2] or in beverages,[3] which can be directly spotted on the preadsorbent after appropriate dilution to provide sample scan areas that are bracketed by standard areas.

The method is much simpler and faster than many reported earlier using impregnated layers and multiple developments.[3,4] Other advantages of the method include good accuracy and precision, as demonstrated by the validation data above, and the ability to run 15 samples with four standards on a single plate, leading to favorable sample throughput and cost-effectiveness.

The described quantitative method is based on a TLC system reported in a student experiment on sugar separation contained in a biochemistry laboratory manual.[1] It can be used as an experiment in analytical chemistry or biochemistry laboratory courses to illustrate clinical analysis by scanning densitometry. Students are very motivated to learn procedures for analyzing their own blood and to compare results with normal values, and the experiment has been very well received in our experience. In addition to the quantitative results for glucose, standards of other sugars can be chromatographed and students required to calculate TLC parameters such as plate number, k', and resolution.[5]

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